REVIEW

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Mitochondria are the powerhouses of immunity

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Recent evidence indicates that mitochondria lie at the heart of immunity. Mitochondrial DNA acts as a danger-associated molecular pattern (DAMP), and the mitochondrial outer membrane is a platform for signaling molecules such as MAVS in RIG-I signaling, and for the NLRP3 inflammasome. Mitochondrial biogenesis, fusion and fission have roles in aspects of immune-cell activation. Most important, Krebs cycle intermediates such as succinate, fumarate and citrate engage in processes related to immunity and inflammation, in both innate and adaptive immune cells. These discoveries are revealing mitochondrial targets that could potentially be exploited for therapeutic gain in inflammation and cancer.

The hypothesis of endosymbiosis dates the origin of what were to become mitochondria at 2.5 billion years ago, when a bacterium that used oxygen to convert organic molecules to energy was phagocytosed by an archaebacterium[1]. The current explanation for the persistence of this arrangement is the mutual benefit it offers to both cell types. The eukaryotic cell acquired a way of limiting oxygen, a toxic molecule that causes oxidation, while having a ready source of ATP, the transporter of chemical energy within cells. The aerobic bacteria gained a safe, nutrient-rich haven. The phagocytosed bacterium was also a likely source of nutrients, producing molecules for macromolecular synthesis in the eukaryotic cell. Recent discoveries related to the role of mitochondria in immunity provide an additional possible explanation for endosymbiosis: the mitochondria might have represented a source of molecules for host defense for the early eukaryotic cell. For example, reactive oxygen species (ROS) could be used for the digestion of phagocytosed pathogens, or as signals for gene expression. Compelling evidence has been accumulating that in complex multicellular organisms such as mammals, mitochondria have multiple critical roles in immunity. Here we discuss these roles and make the case that mitochondria, in addition to being the powerhouses of the cell, also represent the powerhouses of immunity.

Mitochondrial DNA as a DAMP

The similarity between mitochondrial DNA (mtDNA) and prokaryotic DNA is a key piece of evidence for endosymbiosis. mtDNA is a circular loop that contains a substantial number of CpG islands. It is released by necrotic cells and can be sensed by TLR9, the receptor for CpG DNA[2,3], which in turn leads to activation of the NF-κB signaling pathway and the induction of multiple proinflammatory genes, notably those encoding tumor necrosis factor (TNF; also known as TNF-α) and interleukin 6 (IL-6)[4]. mtDNA can activate the NLRP3 inflammasome[5], thereby driving caspase-1 activation, the processing of pro-IL-1β and pro-IL-18, and pyroptotic cell death. mtDNA may also amplify the activation of NLRP3 (ref. 5) by mitochondrial ROS (mtROS)[6], and can directly bind and activate NLRP3 (ref. 7). Finally, mtDNA activates the STING pathway via cGAS, the enzyme that generates the STING activator cGAMP[8]. This culminates in increased interferon-regulatory factor 3 (IRF3)-dependent gene expression, including induction of type I interferons. mtDNA is therefore capable of activating major innate immune signaling pathways by acting as a signal from the mitochondria (in the form of mtDNA release into the cytosol) to the nucleus, which alerts the cell that significant damage is occurring.

Mitochondria as a platform for innate immune signaling

MAVS is the key signaling protein activated by the viral RNA sensors RIG-I and MDA5 (ref. 9). It activates pathways that regulate the transcription factor NF-κB and IRFs to promote gene expression. Interaction with the outer mitochondrial membrane is essential for MAVS function[10] (**Fig. 1**). mtROS can drive MAVS oligomerization, leading to the production of type I interferon independent of RNA sensing, which suggests that MAVS might be a key sensor of mtROS that acts to promote host defense and inflammation[11]. Furthermore, MAVS associates with NLRP3 and promotes its oligomerization, which leads to caspase-1 activation[12]. Activation of NLRP3 with the synthetic TLR7 ligand imiquimod has recently been shown to occur as a result of the induction of mtROS production from both complex I of the respiratory redox chain and the quinone oxidoreductase NQO2 (ref. 13). This effect was independent of K^+ efflux, which highlights the importance of mtROS in NLRP3 activation. Finally, NLRP3 is regulated not only by mtROS but also by the inner mitochondrial membrane lipid cardiolipin, which is thought to be a relic of the cell membrane of the prokaryotic cell that gave rise to mitochondria. After mitochondrial membrane depolarization, cardiolipin translocates to the outer membrane, where it recruits NLRP3 (ref. 14). This interaction

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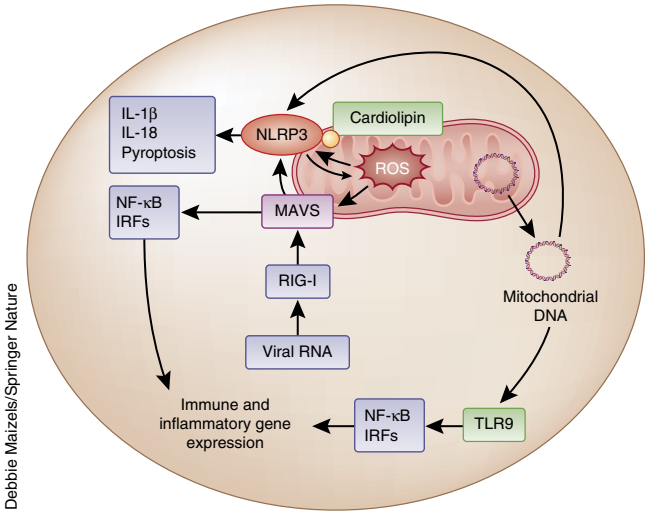

Figure 1 Mitochondria as a signaling hub. MAVS is activated by the viral RNA sensor RIG-I and promotes inflammatory and immune gene expression via NF-κB and IRFs. It signals from the mitochondrial outer membrane. mtROS are also able to activate MAVS independently of RNA. The NLRP3 inflammasome also signals from the outer mitochondrial membrane, and requires cardiolipin, which is extruded to the outer membrane when mitochondria are depolarized. Similar to MAVS, NLRP3 also responds to mtROS and can cause mitochondrial damage that promotes the generation of additional ROS. NLRP3 drives the production of IL-1β, IL-18 and pyroptosis. Mitochondrial DNA can also activate NLRP3, and is also sensed by TLR9, which leads to immune and inflammatory gene expression. Finally, MAVS promotes oligomerization of NLRP3 at the mitochondria. Therefore, three major innate immune pathways—RIG-I/MAVS, NLRP3 and TLR9—are all dependent on mitochondria.

is essential for NLRP3 activation, and suggests that mitochondria could function as a signaling hub for the activation of innate immunity. NLRP3 activation also leads to mitochondrial damage and mtROS production, which suggests a possible feedback loop between NLRP3 and mitochondria[15] (**Fig. 1**). Mitochondria are therefore critical for signaling by three major innate immune signaling pathways: RIG-I/MAVS, NLRP3 and TLR9.

OXPHOS in macrophages, DCs and T cells

Metabolic events in mitochondria are also proving to have profound effects on immunity. The major function of mitochondria is to generate ATP through the process of oxidative phosphorylation (OXPHOS). This multistep pathway leads to the generation of 32 ATP molecules per glucose molecule and is used by quiescent cells in normoxia. Glycolysis, which converts glucose to pyruvate and then generates lactate, was historically shown to occur during hypoxia. ATP production by glycolysis is faster than that by OXPHOS, but the yield is lower[16]—two molecules of ATP per glucose molecule. Both glycolysis and mitochondrial metabolism have an effect on the immune response.

OXPHOS is differentially regulated in M1 (or classical, that is, activated by lipopolysaccharide (LPS) or LPS plus IFN-γ) and M2 (or alternative, meaning IL-4-activated) macrophages, and this is associated with their varying roles in the immune response. M1 macrophages are important in the clearance of microbial infections and are more inflammatory. M2 macrophages have roles in the regulation of inflammation and tissue repair[17]. Stimulation of dendritic cells (DCs) and M1 macrophages is known to decrease OXPHOS, with a concomitant increase in glycolysis and activity in the pentose phosphate pathway[18,19]. Mitochondrial collapse (meaning a decrease in ATP production) occurs in DCs and macrophages after stimulation with LPS and other pathogen-associated molecular patterns as a consequence of nitric oxide (NO) production from arginine[20]. NO inhibits mitochondrial respiration by nitrosylating iron–sulfur-containing proteins, including complex I, complex II and complex IV of the electron-transport chain (ETC), thereby inhibiting electron transport and subsequent ATP production[21]. One outcome of the decrease in ATP production via OXPHOS in LPS-activated macrophages is the repurposing of mitochondria to generate mtROS, signaling molecules that are required for the generation of an appropriate immune response[22], from complex I (ref. 17). It is important to note that LPS-treated macrophages require both glycolysis and complex I activity to generate an appropriate immune response. Thus, inhibition of glycolysis with 2-deoxyglucose[19] and limitation of complex I activity with metformin[23] both impair the induction of IL-1β in response to LPS.

Unlike DCs and inflammatory macrophages, adaptive immune cells do not shut down OXPHOS. The metabolism of T cells varies with effector function and developmental state[24]. Naive T cells metabolize glucose, amino acids and fats to drive the Krebs cycle and OXPHOS, which highlights the importance of respiring mitochondria for T cell function[24–26]. Quiescent naive T cells are activated by ligation of the cell-surface T cell receptor (TCR), in combination with costimulation of other cell-surface receptors (e.g., CD3 and CD28), thus leading to rapid proliferation and differentiation. These processes are characterized by an increase in glycolytic flux[27,28]. Enhanced glycolysis also provides biosynthetic precursors required for proliferating cells or cells with a prodigious biosynthetic capacity, such as T cells activated to produce cytokines. Furthermore, NADH and the α-ketoacid pyruvate, which are produced during glycolysis, are essential drivers of the Krebs cycle. Mitochondrial metabolism increases and is sufficient to support T cell activation even in the absence of glucose[29,30]. Bypassing glycolysis by costimulating T cells in glucose-free culture with pyruvate (to directly fuel the tricarboxylic acid cycle) increases the expression of the T cell activation markers CD25 and CD69. Mitochondria undergo drastic changes after T cell activation, with mitochondrial mass, number, area and amount of mDNA all increasing[31]. These parameters are regulated by multiple signaling pathways, including the activation of protein kinase C and calcium signaling, which are coordinately induced after T cell activation. Uncoupling of either of these pathways illustrates their distinct yet complementary roles in T cell activation[32], and suggests a model whereby mitochondrial mass and OXPHOS are quickly increased to energetically support increased cell size and proliferation. The proteins 5′-AMP-activated kinase (AMPK) and mammalian target of rapamycin (mTOR) have complex roles in this process. It has been suggested that AMPK negatively affects mitochondrial biogenesis via inhibition of mTOR, although this was investigated during the second wave of AMPK activation that occurs approximately 18 h after TCR stimulation, rather than during the first wave of AMPK activation, which occurs within minutes of stimulation[32]. The mitochondrial proteome is significantly remodeled in activated T cells, thus giving rise to a distinct population of mitochondria with a specialized function—namely, folate-mediated one-carbon metabolism—to support T cell proliferation and survival[31]. Various mitochondrial proteins are induced to different extents after T cell activation, with the amount of some mitochondrial proteins increasing two-fold compared to that in naive T cells, whereas others are enhanced 200-fold.

Metabolites in the surrounding microenvironment, derived from non-immune cells, can also affect the ability of immune cells to carry out glycolysis and OXPHOS, and alter their phenotype. In the tumor

microenvironment, lactic acid produced by tumor cells can polarize tumor-associated macrophages to an M2-like phenotype by a mechanism that depends on the transcription factor HIF-1α, and such polarization is likely to promote cancer progression[33]. Tumors deficient in the gene encoding pyruvate kinase M2, an isoform of the glycolytic enzyme pyruvate kinase, which have lower concentrations of lactic acid *in vivo*, show decreased expression of the M2 marker arginase and are significantly smaller compared with tumors with normal gene expression[33]. Mesenchymal stromal cells (MSCs) can alter the phenotype and function of DCs by reprogramming their metabolism in a lactate-dependent manner, an effect that is abrogated in the presence of a lactate dehydrogenase inhibitor[34]. Lactate production by MSCs prevents the differentiation of monocytes into DCs, and it also promotes M2-like properties in monocytes and increases their antigen-presentation capacity, favoring the polarization of $CD4^+$ T cells toward a type 2 helper T cell (T_H2 cell) phenotype[34]. Lactate secretion from MSCs also decreases OXPHOS and mitochondrial function in differentiating DCs. This observation is somewhat puzzling, because M2 macrophages favor OXPHOS; however, lactate production may also induce a HIF-1α-dependent increase in glycolysis, although this was not examined[34].

Mitochondrial dynamics regulate OXPHOS and immunity

"Mitochondrial dynamics" refers to changes in mitochondrial size, shape and localization, as well as the mechanisms that regulate these processes[35]. Mitochondria undergo both fusion and fission processes, and this is evident in T cells (**Fig. 2a**). Naive T cells have fragmented, round mitochondria[31]. Memory T cells (T_m cells) exhibit increased total mitochondrial mass and have elongated mitochondria as a result of decreased fission, whereas effector T cells (T_{eff} cells) have increased fission and more punctate mitochondria with looser cristae[36]. Naive T cells cultured in the presence of the pro-elongation compounds M1 (a fusion promoter[37]) and Mdivi1 (a fission inhibitor[38]) during differentiation display enhanced respiratory capacity. The tighter cristae structure and increased spare respiratory capacity of T_m cells compared with T_{eff} cells is dependent on optic atrophy 1 (Opa1), which is responsible for inner mitochondrial membrane fusion, but not on mitofusin 1 (Mfn1) or Mfn2, both of which mediate outer mitochondrial membrane fusion[36]. These characteristic differences between the two cell types may be central to their function. Fused, elongated mitochondria tend to have efficient ETC supercomplex formation and OXPHOS[39,40], which may assist in cell survival in times of stress[41]. Fission generates fragmented mitochondria with increased ROS production[42], which may be important during T_{eff} cell activation, and enhanced mitophagy[43].

Cristae organization also affects T cell metabolism. Looser cristae may lead to the dissociation of ETC supercomplexes[44,45]. ETC supercomplexes are conserved across species, but their functional importance is not yet clear; they could be involved in the maintenance of efficient respiration, regulation of mtROS production, protein stability or regulation of interactions between individual ETC complexes, or they might have as yet unknown roles[46]. ETC supercomplexes may facilitate efficient electron transfer along the respiratory chain, thus reducing electron leak, which can generate mtROS. Disruption of such supercomplexes could result in less efficient ETC and OXPHOS activity, and increased mtROS production. This could assist T cell activation and proliferation[36]. Lingering electrons might also lead to increased NADH expression. T_{eff} cells increase glycolysis and export lactate to regenerate NAD^+ from NADH in the cytosol, and thus restore redox balance within the cell. Opa1-mediated cristae tightening

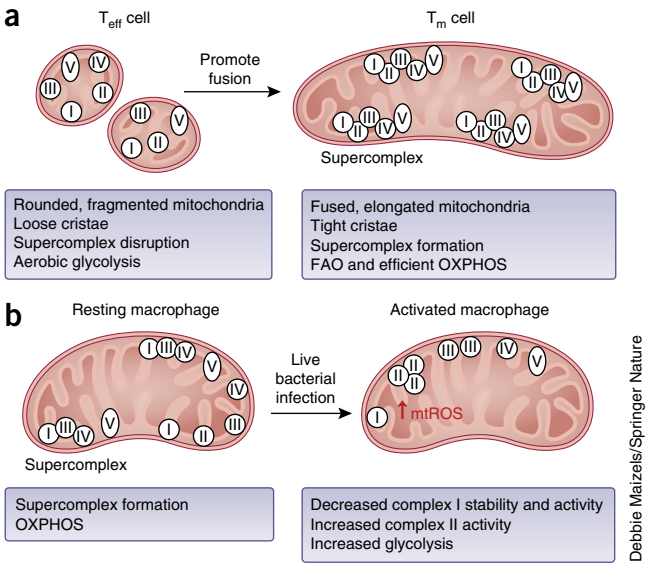

Figure 2 Mitochondrial dynamics regulate immune-cell function. (**a**) T_{eff} cells are characterized by small, round, fragmented mitochondria with loose cristae organization, which may hinder supercomplex formation and will have less efficient electron transport and OXPHOS compared with T_m cells. Enforced mitochondrial fusion promotes a T_m phenotype, with increased FAO and OXPHOS. This may be a result of changes to mitochondrial dynamics, with T_m cells exhibiting fused, elongated mitochondria with tight cristae that facilitate efficient supercomplex formation and OXPHOS. (**b**) Supercomplex organization may also have a role in macrophage function. In resting macrophages, ETC complexes are associated in supercomplexes (also known as respirasomes), which facilitate OXPHOS. Macrophage activation—for example, by live bacteria—disrupts these supercomplexes, particularly by destabilizing complex I. This leads to decreased complex I activity but enhanced complex II activity, as well as increased mtROS production. This ETC disruption is accompanied by an increase in glycolysis.

may promote more efficient OXPHOS, thereby preventing the shift to glycolysis and maintaining metabolic quiescence in T_m cells.

The endogenous complex I inhibitor MCJ, a member of the DnaJ family of chaperones, dampens mitochondrial respiration in $CD8^+$ T cells by disturbing ETC supercomplex formation via an inhibitory association with complex I (ref. 47). MCJ-deficient $CD8^+$ T cells show increased supercomplex assembly, OXPHOS and mitochondrial ATP production, which in turn results in enhanced IFN-γ and IL-2 secretion, but not enhanced proliferation or gene expression of activation markers[47]. There is no increase in mtROS expression, a signal important for T cell proliferation and activation, in MCJ-deficient $CD8^+$ T cells[48]. Instead, the increased amounts of IFN-γ and IL-2 in these cells depend on enhanced production of ATP. Microdomains of OXPHOS-derived ATP, which might be used for cytokine secretion, were identified in MCJ-deficient $CD8^+$ T cells[47]. Thus, mitochondrial ATP can have a signaling role and can be secreted from T cells to act in an autocrine stimulatory fashion[49]. MCJ deficiency in $CD8^+$ T cells induced increased immunity in a mouse model of influenza infection[47].

Mitochondrial morphology is also functionally important in $CD4^+$ T cells. Deficiency of the mitochondrial transcription factor TFAM drastically disrupts mitochondrial morphology in $CD4^+$ T cells, resulting in impaired cristae organization[50]. ETC function is compromised in TFAM-deficient $CD4^+$ T cells, as indicated by reduced amounts of complexes I and III, but not complex II, and stronger impairment of electron transport from NADH than from $FADH_2$. Respiration, ATP production and fatty acid oxidation (FAO) are also reduced.

Tfam$^{-/-}$ T cells are more inflammatory, with increased transcription and secretion of IFN-γ and IL-6, but reduced amounts of the immunosuppressive cytokine IL-10.

The inner mitochondrial membrane serine/threonine phosphatase PGAM5 has recently been identified as the second known mammalian phosphohistidine phosphatase. It is associated with mitochondrial fragmentation and fission. It can be cleaved, probably by an intramembranous protease in the mitochondria, to generate a cytosolic pool of PGAM5. The cytosolic form of PGAM5 negatively regulates TCR-stimulated calcium influx and proinflammatory cytokine production in CD4$^+$ T cells, and graft-versus-host disease was shown to be exacerbated in *Pgam5*$^{-/-}$ mice[51]. PGAM5 inhibits TCR signaling by dephosphorylating a histidine residue on the potassium channel KCa3.1. These observations ascribe an intriguing function to mitochondrial PGAM5.

Mitochondrial dynamics are less well characterized in innate immune cells, although a recent study showed that stimulation of macrophages with live bacteria alters ETC architecture by decreasing supercomplex formation in a ROS-dependent manner[52], and that such ETC adaptations are critical for the generation of an appropriate response to bacterial infection (**Fig. 2b**). Alterations to mitochondrial dynamics, including fusion/fission, ETC architecture and cristae organization, regulate the metabolic activity and immune function of both innate and adaptive immune cells.

Mitochondrial ROS control adaptive immune-cell activation

ROS are a consequence of mitochondrial disruption in both M1 macrophages and T_{eff} cells. Complexes I and III of the ETC serve as the major sites of ROS production. ROS are directly antibacterial, and also signal to drive the production of inflammatory cytokines. T cell activation induces a rapid increase in mtROS production, and blocking this process antagonizes IL-2 production by T cells. T cells that lack the complex III component RISP have decreased expression of the activation markers CD25 and CD69 (ref. 30). TCR signaling leads to the release of calcium stored in the endoplasmic reticulum, which can be taken up by mitochondria to drive Krebs cycle enzyme activity, thereby increasing the amounts of NADH and Krebs cycle intermediates such as succinate. Mitochondrial calcium influx contributes to mtROS generation in T cells, and succinate drives mtROS generation in macrophages. Mitochondrial membrane potential is tied to oxidative stress[53]. A reduction of membrane potential in CD8$^+$ T cells decreases ROS production and increases the expression of the ROS detoxifiers catalase, glutathione peroxidase 4 and superoxide dismutase (SOD1 and SOD2)[53]. T_H1 cells and IL-17-producing helper T cells (T_H17 cells) with high membrane potential have increased expression of IFN-γ or IL-17A and IL-17F, respectively[53].

An alternative source of mtROS in T cell activation is the innermitochondrial membrane enzyme GPD2 (ref. 54). During T cell activation, glycolysis supplies excess glycerol-3-phosphate, which is imported into mitochondria for lipid biosynthesis. GPD2 oxidizes glycerol-3-phosphate to dihydroxyacetone phosphate, thereby providing electrons to hyper-reduce the Q pool in the inner mitochondrial membrane[54]. GPD2 could directly produce ROS, and additionally the hyper-reduction of the Q pool could support ROS production at other ETC sites, such as complex I. GPD2 depletion by small interfering RNA has been shown to inhibit mtROS production during T cell activation and to decrease IL-2 expression[54].

mtROS also function as a signal in B cell activation. B cell activation by antigen and helper T cells induces processes such as somatic hypermutation, to diversify the B cell receptor for antigen, and class-switch recombination (CSR), which enables B cells to express a particular type of immunoglobulin. Ligation of the BCR stimulates calcium release into the cytoplasm, which promotes ROS production[55]. Stimulation for 1 d with LPS and IL-4 or with anti-CD40 and IL-4 generates a population of B cells with increased mitochondrial mass, membrane potential and mtROS production compared with naive B cells. Undifferentiated cells within this population undergo CSR after differentiation. mtROS may act to induce CSR by inhibiting the synthesis of heme, a molecule that decreases CSR by antagonizing BACH2 (ref. 56), a key transcription factor for CSR[57].

Taken together, these observations indicate that T and B cells require ROS production for the generation of an appropriate immune response. Innate immune cells are similarly reliant on ROS production, with Krebs cycle intermediates such as succinate, citrate, fumarate and itaconate emerging as important regulators of the production of this noxious agent, as well as other events in immunity, as will now be discussed. Overall, mtROS, originating from a variety of mitochondrial sources, are key signaling molecules for immune-cell function.

Succinate is a regulator of inflammation and disease

The Krebs cycle generates a number of metabolites with important inflammatory signaling functions. These metabolites accumulate in M1 macrophages because of the existence of a number of break points in the Krebs cycle. A break at the enzyme succinate dehydrogenase (SDH), which converts succinate to fumarate and is complex II of the ETC[58], is associated with an accumulation of succinate, a metabolite with proinflammatory activity and roles in immunity (reviewed in ref. 59). Succinate oxidation by SDH, and subsequent ROS production from complex I in a process termed reverse electron transport (RET), leads to HIF-1α activation and HIF-1α-dependent gene expression; a notable example of a gene affected in this way is *IL1B*, which encodes IL-1β (ref. 60). Succinate also limits the production of anti-inflammatory cytokines, particularly of IL-10. Inhibition of SDH with dimethylmalonate (DMM) profoundly inhibits LPS-induced mtROS generation and the expression of IL-1β and a range of proinflammatory genes in macrophages, and boosts the expression of IL-10 and anti-inflammatory genes[54]. DMM administration *in vivo* in mice treated with LPS results in decreased IL-1β expression and a boost in IL-10 expression systemically[54]. LPS treatment boosts the mitochondrial membrane potential in macrophages; this is dependent on increased glycolytic ATP production. Limiting of LPS-induced membrane hyperpolarization, which is required for RET, inhibits IL-1β induction in response to LPS in macrophages. The induction of IL-1β by LPS and succinate is also impaired in macrophages that express the enzyme alternative oxidase (AOX), which limits RET[54]. All of these observations[54] not only confirm a role for mitochondria-derived succinate as a critical proinflammatory mediator, but also shed light on the mechanism by which LPS drives ROS production, and provide further rationales for why macrophages favor aerobic glycolysis and decrease OXPHOS in response to LPS. These metabolic alterations repurpose mitochondria from ATP synthesis to ROS production to promote a proinflammatory state. An antibacterial role of SDH was also suggested by the observation that the inhibition of SDH during *Escherichia coli* or *Salmonella enterica* infection rendered mice more susceptible to infection[52]. Taken together, these findings identify SDH as a key control point in inflammation and host defense (**Fig. 3**).

A role for RET in metabolic signaling has been described in a variety of settings, which suggests that RET might be a general phenomenon for ROS generation in different biological contexts. RET has been demonstrated in ischemia/reperfusion injury[61], mtROS production during aging in *Drosophila*[62], and hypoxia sensing in the carotid body, a cluster of cells near the bifurcation of the carotid

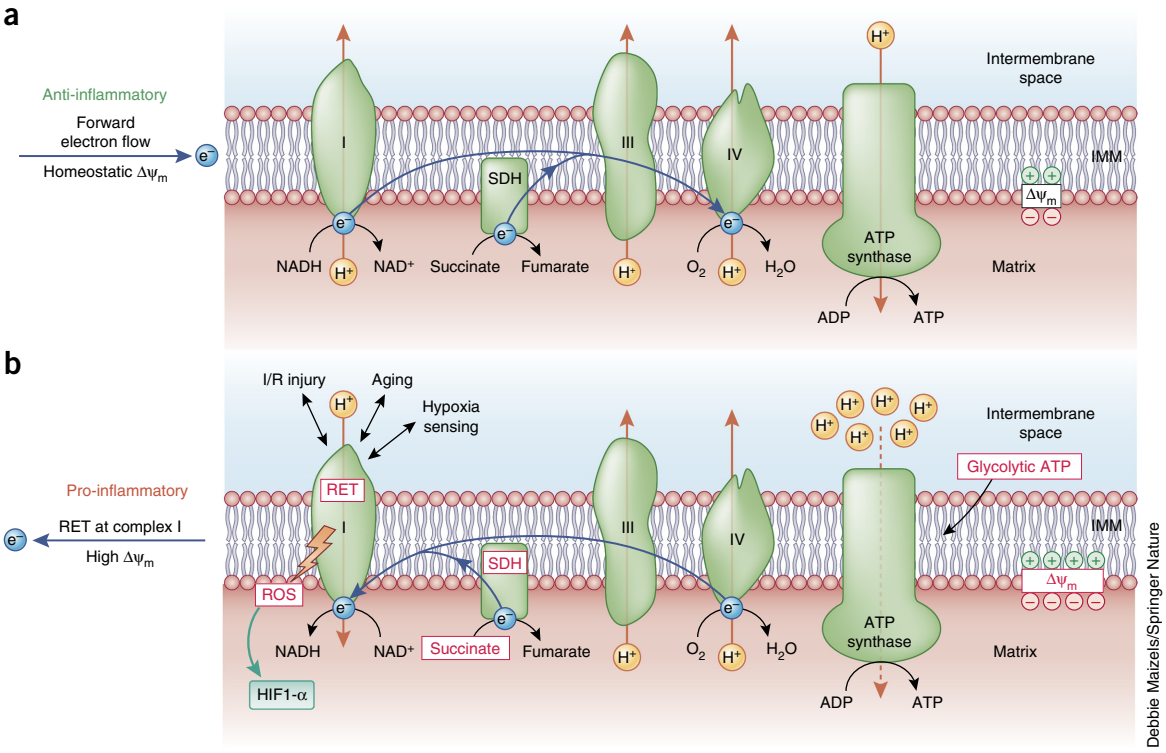

Figure 3 Succinate as a proinflammatory signal acting via SDH and RET in complex I. In M1 macrophages, OXPHOS is uncoupled, and there is an increase in the mitochondrial membrane potential ($\Delta\Psi_m$). Glycolytic ATP is required to maintain mitochondrial integrity. Succinate accumulates and is oxidized by SDH. This leads to RET in complex I, which generates ROS and thus promotes the expression of inflammatory genes. Simultaneously, there is a decrease in the expression of anti-inflammatory genes, which leads to a net inflammatory state. If SDH is inhibited, these processes are altered such that there is a shift to the anti-inflammatory (and possibly homeostatic) state. RET has also been implicated in the pathogenesis of ischemia/reperfusion (I/R) injury in aging in *Drosophila*, and in hypoxia sensing in the carotid body. IMM, inner mitochondrial membrane.

artery that detects oxygen levels[63]. Furthermore, metformin, which inhibits complex I, decreases IL-1β expression in response to LPS in macrophages[23]. Also of interest is the recent identification of TLR4 as a driver of ROS production and subsequent systemic inflammation in mice deficient in the complex I subunit Ndufs4 (ref. 64), which further emphasizes the modulation of complex I by TLR4. Overall, therefore, succinate action via RET provides a mechanism for mtROS generation in LPS-activated macrophages, with RET also having a role in the generation of mtROS in other settings.

Itaconate regulates SDH and is antibacterial

Although succinate and SDH activity are essential for the generation of a proinflammatory response in LPS-activated macrophages, prolonged SDH activity is likely to have a detrimental effect on the host by inducing excessive ROS production and hyperinflammation. To control this inflammatory response, macrophages use the metabolite itaconate, which can inhibit SDH function and the proinflammatory response. The amount of itaconate is boosted in LPS-stimulated and M1 macrophages as a result of decreased expression of isocitrate dehydrogenase, as this enzyme diverts citrate away from itaconate[58]. Itaconate is generated by decarboxylation of aconitate (produced from citrate) by the enzyme Irg1, the expression of which is also enhanced in both M1 macrophages and LPS-treated macrophages[58,65,66] (**Fig. 4**). The role of itaconate in macrophage function remained unclear until recently, when a study showed that pretreatment of macrophages with itaconate reduces the LPS-induced production of IL-1β, IL-12p70 and IL-6, as well as iNOS expression and ROS production, most likely by limiting RET as a result of decreased succinate oxidation by SDH[61]. Mice that lack Irg1, and thus do not produce itaconate, have decreased levels of succinate[61], which suggests that in the absence of itaconate, SDH effectively oxidizes succinate to fumarate. Irg1-deficient macrophages show high production of IL-1β, whereas TNF production is unaffected, which suggests that TNF is regulated via a non-mitochondrial-associated mechanism[61]. Inhibition of IL-1β expression with no effect on TNF is similar to what was observed in macrophages treated with DMM to inhibit SDH[54]. Itaconate further limits LPS-induced HIF-1α expression in macrophages[61], which suggests that it may limit the inflammatory response by decreasing the generation of mtROS, possibly derived from succinate-induced RET at complex I.

Itaconate has roles in immunity in addition to its regulation of the proinflammatory response in macrophages. Itaconate inhibits the growth of *S. enterica*, *Mycobacterium tuberculosis* and *Legionella pneumophila* in liquid culture[65,67]. Silencing of *Irg1* (*Acod1*) in mouse macrophages impairs antimicrobial activity during microbial infections[65]. Itaconate inhibits the enzyme isocitrate lyase, a component of the glyoxylate shunt[68], which is an essential metabolic pathway required for the survival of bacteria that use fatty acids or acetate as their primary carbon source, such as *E. coli* and *Yersinia pestis*, the causative agent of the bubonic plague[69]. The glyoxylate shunt is absent in mammals; therefore, rewiring the Krebs cycle in this manner in infected cells would target a bacteria-specific metabolic pathway. Itaconate is thus an intriguing metabolite derived from citrate that seems to have anti-inflammatory and antibacterial effects.

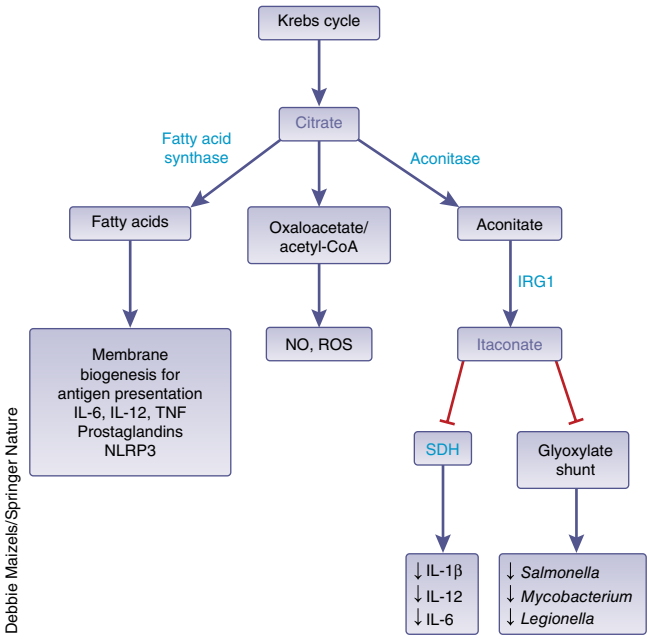

Figure 4 Citrate and itaconate are key regulators of inflammation and antibacterial host defense. Citrate from the Krebs cycle can be converted to itaconate by the enzyme IRG1. Citrate inhibits SDH to block inflammation, and also exerts direct antibacterial effects by acting on the bacterial glyoxylate shunt. Citrate can also be converted to fatty acids via fatty acid synthase; this enzyme is required for the induction of inflammatory cytokines and prostaglandins, as well as for membrane biogenesis for antigen presentation in DCs. Fatty acids can also activate the inflammasome. Citrate generates oxaloacetate and acetyl-CoA, which are needed for NO and ROS production.

Citrate and fatty acid synthesis are proinflammatory

The decreased amount of isocitrate dehydrogenase leads not only to an accumulation of itaconate but also to an accumulation of citrate, which has been shown to drive an inflammatory response in M1 macrophages[19,58] (**Fig. 4**). Citrate is required for fatty acid biosynthesis, and once in the cytosol it is cleaved by citrate lyase into acetyl-CoA and oxaloacetate. The mitochondrial citrate carrier (CIC) is responsible for the transport of citrate from the mitochondria to the cytosol. LPS increases the expression of CIC in macrophages[70], most likely via NF-κB transcriptional regulation, as the CIC promoter contains two NF-κB-binding sites, and CIC is required for LPS-mediated induction of ROS and NO[70], both of which require oxaloacetate metabolism and prostaglandins[70], which require phospholipid synthesis from fatty acids.

Fatty acids derived from citrate and other metabolic pathways are essential for cellular growth and proliferation, and have been implicated in DC activation and the production of proinflammatory mediators in those cells[71]. The incorporation of glucose-derived carbon into lipids in LPS-stimulated DCs is prevented by C75, an inhibitor of fatty acid synthase[65]. Treatment with C75 impairs the expansion of endoplasmic reticulum and Golgi in response to LPS, and subsequently the ability of DCs to present antigen and to generate proinflammatory cytokines such as IL-6, IL-12 and TNF[65] (**Fig. 4**), which suggests that the generation of fatty acids is essential for an appropriate immune response in these cells. Fatty acid synthesis has also been suggested to regulate NLRP3-mediated caspase-1 activation and the production of IL-1β and IL-18 in macrophages[72].

Citrate and acetyl-CoA control IFN-γ production

Another link to citrate was recently reported to be of relevance to T cells. Balmer *et al.*[73] showed that acetate boosts IFN-γ production in T cells, and they propose that this might occur via citrate production. Exogenous acetate contributes to the production of citrate and acetyl-CoA, with the enzyme ATP citrate lyase converting citrate to acetyl-CoA. The acetyl-CoA produced is used to acetylate GAPDH at Lys217, and this is possibly linked to the increased IFN-γ production. GAPDH inhibits IFN-γ production by binding its mRNA transcript[74], and it is possible that acetylation of GAPDH leads to the dissociation of GAPDH from IFN-γ mRNA, thus allowing it to be translated. Increased GAPDH acetylation may occur physiologically, as amounts of serum acetate transiently increase during infection[73]. In addition, an epigenetic link between glycolysis, acetylation and IFN-γ production has also been proposed, implicating histone remodeling of the *Ifng* promoter in the increased transription[75]. Increased amounts of acetyl-CoA resulting from the induction of lactate dehydrogenase were detected in activated T cells[75]. The increase in lactate dehydrogenase promotes the flux through glycolysis that would allow for pyruvate to be made, thus feeding citrate production in the mitochondria. This in turn leads to acetyl-CoA production from citrate by the enzyme ACLY, with the acetyl-CoA being used to boost histone acetylation and transcription of IFN-γ (ref. 76). This occurs independently of GAPDH binding to the 3′ UTR of IFN-γ, although the evidence for this is based on the use of an artificial construct—the bovine growth hormone 3′ UTR—rather than direct manipulation of the *Ifng* 3′ UTR itself. Multiple mechanisms involving acetyl-CoA generated from citrate may therefore exist to regulate *Ifng* expression. Activated T cells may remodel their chromatin, thereby generating *Ifng* mRNA that can be robustly translated, as GAPDH is engaged in glycolysis and no longer binds the *Ifng* mRNA. Then, in glucose-poor conditions, with consequently decreased glycolysis, GAPDH may bind *Ifng* mRNA to prevent its translation, and histone acetylation will also be limited, thus decreasing *de novo Ifng* mRNA synthesis[77]. Therefore, both processes—GAPDH binding to *Ifng* mRNA, and a lack of chromatin remodeling at the gene because of decreased acetylation—may occur when glucose levels are low. These studies reveal the complexity of the ways in which metabolic processes integrate with gene expression (in this case, of *Ifng*).

Metabolites regulate the epigenome in trained immunity

Another target for Krebs cycle intermediates is the α-KG-dependent dioxygenase enzyme family, in particular the enzymes involved in the regulation of histone and DNA demethylation, the α-KG-dependent Jumonji C-domain-containing histone demethylases (JMJDs) and the Ten-eleven translocation (TET) family of 5mC hydroxylases, which have roles in DNA and histone demethylation, respectively. These enzymes are subject to feedback inhibition by succinate, so their activity is dependent on the ratio of α-ketoglutarate (α-KG) to succinate[78,79]. This ratio can therefore remodel the epigenome. One consequence of altering the epigenome at specific genes is innate immune training, whereby innate immune cells acquire a form of immunological memory and can respond more robustly to a second stimulation that is not necessarily related to the first[80]. Epigenetic marks persist after the stimulus that induced them has resolved, and can therefore represent stable signals. At a molecular level, innate immune training involves epigenetic modifications that lead to stronger gene transcription after restimulation, which, unlike adaptive memory, does not involve gene recombination.

Such innate immune training occurs in human monocytes stimulated *in vitro* with β-glucan, a component of *Candida albicans*, and

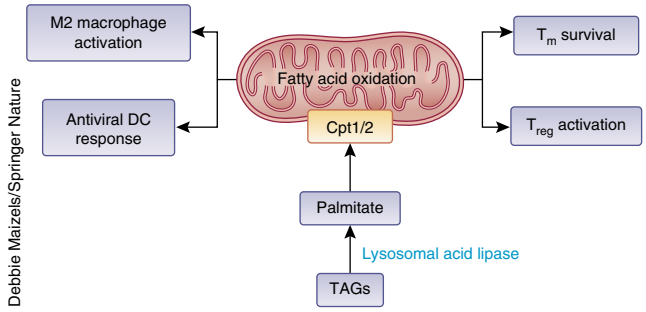

Figure 5 FAO is required for T_m cell, T_{reg} cell, M2 macrophage and antiviral DC responses. Fatty acids generated from triacylglycerol (TAG) by lysosomal acid lipase are taken up by mitochondria and oxidized, with the enzymes Cpt1 and Cpt2 having key roles. This process is essential for the maintenance of T_m cells, the activation of T_{reg} cells and M2 macrophages, and the ability of DCs to limit viral replication.

subsequently treated with LPS, with elevated cytokine production observed after LPS restimulation[81]. The elevated cytokine response in these cells is linked to an increase in the amount of fumarate sufficient to drive the response by activating HIF-1α, thus inhibiting the histone demethylase activity of the demethylating enzyme KDM5 and enhancing trimethylation of histone H3 on lysine 4 (H3K4me3) on the gene encoding TNF[70]. Thus, alterations in monocyte metabolism underpin β-glucan-induced trained immunity, which suggests that metabolic alteration could represent a mechanism for boosting innate immune training[82].

FAO is important for M2 macrophage function

The Krebs cycle is fueled by intermediates generated by other metabolic pathways, such as FAO. In mitochondria, FAO catabolizes fatty acids to acetyl-CoA, NADH and $FADH_2$, which support flux through the Krebs cycle and OXPHOS. This pathway can generate more than 100 ATP molecules from one molecule of palmitate. The rate-limiting step in FAO is the conjugation of fatty acids to carnitine via CPT1, so this enzyme serves as a major control point in the determination of FAO rates.

FAO is differentially regulated in M1 and M2 macrophages. M2 cells have increased FAO[83], which is driven by PGC-1β (ref. 84) in a manner dependent on STAT6 activation in response to IL-4. M2 macrophages also regulate FAO via AMPK[85]. AMPK acts in concert with PPAR-δ to boost FAO in human macrophages[86]. Oxidative metabolism in M2 macrophages has been directly linked to their anti-inflammatory phenotype. Inhibition of FAO in M2 macrophages with the carnitine palmitoyltransferase 1 (CPT1) inhibitor etomoxir limits M2 activation in response to IL-4 (ref. 87). Furthermore, M2 cells engage in a futile cycle of CD36-dependent uptake of triglycerides followed by subsequent lipolysis of those fatty acids by lysosomal acid lipase[87] (**Fig. 5**). This process is important for the elevated OXPHOS observed in these cells. In contrast, etomoxir has no effect on IL-4-induced expression of *Arg1*, *Fizz-1*, *Ym-1* or *Mrc-1*, which suggests that FAO is dispensable for M2 polarization[77]. It is important to note that this last observation was made sooner after IL-4 treatment, which suggests that FAO is required for the sustained induction and prolonged survival of M2 macrophages[88]. Etomoxir has no effect on IL-4-induced M2 polarization in human macrophages, which suggests that there is a difference between mouse and human macrophages[89].

Furthermore, macrophages from mice deficient in CPT2, one of the two mitochondrial membrane enzymes that make up the CPT system, show substantially impaired FAO, yet etomoxir, which is reportedly a specific CPT1 inhibitor, decreases FAO in wild-type cells but not in CPT2-deficient cells[90]. Intriguingly, wild-type and CPT2-deficient mouse macrophages respond similarly to IL-4, which boosts the expression of *Arg1*, *Retnla* and *Mgl2* as readouts for M2 polarization[79]. These data suggest that FAO may be dispensable for M2 activity and that etomoxir, which effectively decreases the expression of M2 markers in both wild-type and CTP2-deficient macrophages, may have an additional, as yet undetermined role in M2 macrophage polarization and activity.

FAO has also been implicated in plasmacytoid DC (pDC) function. Stimulation of pDCs with CpG or recombinant IFN-α upregulates FAO and OXPHOS[80], and this effect is abrogated in mice deficient in interferon receptor 1. This metabolic switch is critical for pDC activation and the induction of an antiviral response[91]. Etomoxir decreases CpG-induced OXPHOS and the production of IFN-α, TNF and IL-6 in pDCs[80]. A shift toward FAO is vital for an appropriate immune response, as demonstrated by the fact that pretreatment with etomoxir increases viral load in the liver and spleen of mice infected with choriomeningitis virus; this suggests that FAO induction by type I interferon may limit the amount of free fatty acids that can be used for viral replication. These data suggest that FAO is an anti-inflammatory metabolic pathway that acts to both enhance M2 function and limit inflammatory macrophage function.

Fatty acid metabolism is a vital process for T cell function

Fatty acid metabolism is similarly vital for T cell function, particularly for T_m cells. Boosting FAO with the AMPK activator metformin, which is widely used to treat type 2 diabetes, enhances T_m cell development after immunization with *Listeria monocytogenes* ovalbumin (OVA) and improves the efficacy of an experimental anticancer vaccine, providing increased immunity against EL4–OVA tumor cells[92]. IL-15, a cytokine that promotes $CD8^+$ T_m cell development and maintenance, promotes mitochondrial biogenesis and the expression of an isoform of CPT1 (CPT1a), the rate-limiting enzyme of FAO. Thus, IL-15 increases the spare respiratory capacity (SRC) and oxidative metabolism of $CD8^+$ T_m cells[93]. SRC is the extra. capacity available in cells to produce energy in response to increased stress or work[94–96]. T_m cells, but not T_{eff} cells, have substantial SRC, a property associated with survival. Etomoxir impairs SRC and the oxygen consumption rate of T_m cells, but not T_{eff} cells, and decreases T_m cell survival[93]. Conversely, retroviral enhancement of CPT1a expression increases SRC and T_m cell survival.

Fatty acids in T_m cells come from internal lysosomal stores, rather than an extracellular source. Compared with T_{eff} cells, T_m cells have reduced CD36 surface expression (necessary for fatty acid uptake). Furthermore, unlike T_{eff} cells, T_m cells engage in *de novo* lipogenesis and store lipids in lysosomes[97]. Like M2 macrophages, T_m cells engage in a futile cycle of lysosomal-acid-lipase-mediated lipolysis to liberate free fatty acids from storage followed by robust FAO in mitochondria, possibly as a means to ensure that they have a continuous lipid supply for FAO, regardless of the extracellular lipid content[29] (**Fig. 5**). IL-7, which supports the survival of $CD8^+$ T_m cells, increases triglyceride synthesis by selectively boosting the expression of the glycerol channel AQP9 in T_m cells, but not in T_{eff} cells or naive T cells[98]. AQP9-deficient memory T cells have poor survival owing to decreased glycerol import and consequently reduced triglyceride synthesis[98]. Such decreased triglyceride levels may limit FAO, thus reducing T_m cell survival. Indeed, amounts of free fatty acids are lower in AQP9-deficient $CD8^+$ T cells[98] than in wild-type cells, which results in limited fuel availability for FAO, and reliance on glycolysis[98]. Overexpression of genes involved in triglyceride synthesis in AQP9-deficient mice

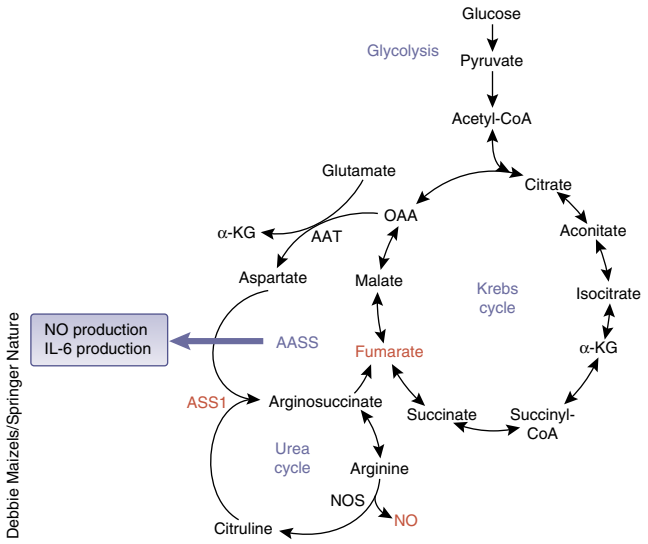

Figure 6 The aspartate–arginosuccinate shunt and macrophage activation. In macrophages, the aspartate–arginosuccinate shunt (AASS) is a key pathway that replenishes the Krebs cycle at fumarate. Arginosuccinate synthase (ASS1) is strongly upregulated in LPS-activated macrophages. This leads to a boost in levels of citrate and succinate, which have key functions in inflammation. The AASS is required for the induction of IL-6, and also for NO production via the interface with the urea cycle. OAA, oxaloacetate; AAT, aspartate aminotransferase.

restored T_m cell populations to numbers similar to those in wild-type mice. IL-7 promoted *AQP9* expression, TAG synthesis and survival in human T_m cells[98], which is of particular significance, as IL-7 may have potential as a vaccine adjuvant[99,100].

Though FAO and SRC are important for the function of central memory T cells, glycolysis can support the function of effector memory T cells (T_{EM} cells). The E3 ubiquitin ligase von Hippel–Lindau (VHL) protein degrades HIF-1α under normal oxygen tension. VHL-deficient T cells, which have increased glycolysis and decreased oxidative metabolism compared with wild-type cells, are long-lived, express markers of T_{EM} cells, and can clear secondary infection by lymphocytic choriomeningitis virus[101]. VHL-deficient T cells do maintain some mitochondrial metabolism, however, and it is possible that T cells *in vivo* are adaptable and may use various metabolic pathways. For example, it is less likely that T_m cells would rely on glycolytic metabolism in glucose-poor environments such as tumors[102]. Finally, regulatory T cell (T_{reg} cell) differentiation requires FAO[103]. Overall, although FAO is important in both T_m and T_{reg} cells, there is some adaptability.

Glutamine, arginine and the aspartate arginosuccinate shunt

Amino acids also fuel the Krebs cycle and contribute to the maintenance of amounts of intermediates of this pathway such as α-KG, succinate and fumarate. In M1 macrophages, increased flux through the aspartate–arginosuccinate shunt feeds the Krebs cycle at fumarate, replenishing the cycle after the break point that occurs in M1 macrophages at SDH[58] (**Fig. 6**). The aspartate–arginosuccinate shunt connects the Krebs cycle to the urea cycle, a pathway involved in the production of NO. Intriguingly, arginosuccinate synthase, the rate-limiting enzyme for arginine synthesis, is strongly upregulated in M1 macrophages[58], and arginine can boost OXPHOS and limit ROS production in tumor cells[104]. Inhibition of the aspartate–arginosuccinate shunt enzyme Got1 decreases NO production, iNOS expression and IL-6 production, markers characteristic of M1 macrophages[58]. This decrease in NO production is coupled to an increase in OXPHOS and glycolysis comparable to that in untreated or M0 macrophages[58].

Glutamine can also be metabolized to feed the Krebs cycle. Increased glutamine metabolism through anaplerosis of α-KG into the cycle contributes to the elevated amounts of succinate observed after LPS treatment of macrophages[19]. Increased glutamine uptake also replenishes the Krebs cycle when citrate is used for the production of itaconate and for lipogenesis, and may also modulate macrophage lipotoxicity[105]. Glutamine deficiency reduces lipid-induced lysosomal dysfunction, inflammasome activation and cell death in macrophages, and also boosts autophagy[105].

Amino acids modulate mitochondrial metabolism in T cells

Amino acid metabolism has also been implicated in the induction of OXPHOS in T cells, which consequently increases T cell survival and antitumor function[106]. L-arginine supplementation during the activation of naive T cells promotes OXPHOS and limits IFN-γ production. Intriguingly, supplemented T cells have an increased capacity to produce IFN-γ after restimulation, which implies that the increased arginine imparts a memory-like phenotype in T cells[106]. Indeed, T cells supplemented with L-arginine expressed T_m cell markers and demonstrated enhanced survival compared with controls in an *in vivo* antitumor model[106].

SHMT2 catalyzes the interconversion of the amino acids serine and glycine in the mitochondria. This enzyme is massively induced during the activation of naive $CD4^+$ T cells[31]. Metabolic tracing analysis indicates that serine is a key donor of one-carbon units for one-carbon metabolism in activated $CD4^+$ T cells, a pathway that metabolizes folate to achieve *de novo* purine biosynthesis[107]. $CD4^+$ T cell activation generates a population of mitochondria with enhanced one-carbon metabolism, and impairment of mitochondrial one-carbon metabolism decreases $CD4^+$ T cell survival and the abundance of antigen-specific T cells[31]. It is not clear whether several distinct populations of mitochondria exist within the activated cell. SHMT2-deficient activated T cells have decreased purine levels compared with wild-type controls, as well as increased DNA damage and impaired survival. The addition of a combination of one-carbon units (formate) and the antioxidant N-acetyl cysteine rescues cell death[31]. These observations indicate that one-carbon and serine metabolism are important for $CD4^+$ T cell survival and antigen-specific $CD4^+$ T cell abundance *in vivo*.

Glutamine is a key fuel source for T cells and, as in macrophages, is anaplerotically metabolized in the mitochondria to replenish Krebs cycle intermediates that are exported, such as citrate[108]. T cell activation induces a transient increase in amounts of the glutamine transporters SNAT1 and SNAT2 (ref. 109). Glutamine withdrawal inhibits oxygen consumption in T_{eff} cells and reduces ATP levels, which indicates that glutamine fuels mitochondrial metabolism in T cells. Glucose restriction induces genes involved in glutamine metabolism, thus implicating glutamine as an energy source under glucose deprivation[110]. Indeed, stable isotope tracer analysis showed that T_{eff} cells starved of glucose have increased amounts of glutamine-derived glutamate and pyruvate, which allows these cells to maintain their Krebs cycle activity[110]. B cells also engage in increased glutamine metabolism during the process of clonal expansion[111,112].

Intriguingly, amino acid transporters, as well as amino acids themselves, can be asymmetrically distributed after the first division of an activated T cell, as can the cell-cycle regulator c-Myc and the metabolic checkpoint kinase complex mTORC1, which drive metabolic reprogramming after T cell activation. The daughter cell proximal to

Box 1 Outstanding questions

- What are the roles of mitochondrial fission and fusion and of cristae remodeling in innate immune cells? Are they linked to the production of ROS in these cells, possibly through alteration of RET?
- Do metabolites have additional, undiscovered roles in immunity? For example, can succinate mediate additional signaling roles via ligation of the succinate receptor SUCRN1 in macrophages, and are there other metabolite-sensing G-protein-coupled receptors yet to be discovered? Could metabolites act as danger signals to activate NLRP3? Will acetyl-CoA boost histone acetylation and transcription of other cytokines, or in other immune cells?
- Can metabolites such as succinate and itaconate serve as biomarkers of disease? Do they contribute to disease pathogenesis? Could DMM and rotenone serve as possible therapeutics without toxic side effects?
- Is FAO required for M2 activity, and does CPT1 have roles outside of FAO in M2 polarization and activity? Could this explain the purpose of the futile cycle of fatty acid synthesis followed by oxidation?
- Can pathogens and host microbes generate metabolites that influence the immune response?
- Are other innate immune cells, such as innate lymphoid cells, natural killer cells and granulocytes, subject to metabolic repurposing?

the antigen-presenting cell expresses higher levels of the amino acid transporters CD98 and SLC1A5 and of amino acids compared with the more distal daughter cell, and it contains mTORC1 and c-Myc protein. Increased amino acid levels are sensed by the Rag GTPase RagC, which recruits mTORC1 to the lysosome, thus facilitating its activation. Increased colocalization between mTORC1 and the lysosome also was observed in proximal daughter cells. This differential distribution of mTORC1 promotes c-Myc translation in proximal daughter cells, which are more glycolytic and more likely to develop into T_{eff} cells. Furthermore, proximal daughter cells with higher mTORC1 activity exhibit increased glycolysis and expression of effector molecules, whereas those with lower mTORC1 activity have enhanced lipid metabolism, SRC and survival, which are features of the memory phenotype[113]. Thus, asymmetric division of activated T cells is a means of regulating their metabolic profile and function, and as such may be a means of driving activated T cells toward either an effector phenotype (in the case of the cell proximal to the antigen-presenting cell) or a memory phenotype (in the case of the more distal cell).

Mitochondria: a new therapeutic target?

One therapeutic option that has emerged from studies on macrophages is the conversion of M1 inflammatory macrophages to M2 anti-inflammatory cells for therapeutic gain. Metformin and rotenone might induce this switch. Metformin was identified as an anti-inflammatory agent that decreases LPS-induced pro-IL-1β and ROS while boosting IL-10 (ref. 23). It has been speculated that metformin targets complex I of the ETC to decrease RET, ROS and pro-IL-1β production[58]. Inhibition of SDH with DMM may also provide a therapeutic benefit by limiting ROS production and proinflammatory responses, and boosting the anti-inflammatory response. If the reprogramming were to be sustained, possibly by epigenetic changes downstream of the metabolic changes, it could offer the possibility of inducing remission in chronic inflammatory diseases. It is likely that targeting other key components required for M1 and M2 polarization—for example, inhibition of fatty acid synthesis, glycolysis and the AASS—or boosting FAO or OXPHOS could be beneficial.

T cell immunotherapies, including checkpoint-inhibitor blockade, chimeric antigen receptor (CAR) T cells and adoptive cell transfer, are clinical techniques that are widely used to combat leukemia, lymphomas and solid tumors. Insight into the metabolic characteristics of T cells associated with successful antitumor responses is therefore of benefit. In chronic infections and cancer, persistent ligation of the T cell coinhibitory molecule PD-1 promotes T cell exhaustion[114,115]. This condition is associated with reduced mitochondrial respiration and glycolysis, and exhausted T cells have fused, depolarized mitochondria and increased ROS compared with T_{eff} cells. PD-1 establishes this metabolic dysregulation by negatively regulating the transcription coactivator PGC-1α, thereby antagonizing mitochondrial biogenesis. Overexpression of PGC-1α in T cells transferred to mice with established tumors led to increased mitochondrial mass, and the T cells had antitumor immunity[116]. PGC-1α may therefore represent a therapeutic target for reviving exhausted $CD8^+$ T cells in diverse settings[117].

Immunotherapy involving the adoptive transfer of tumor-specific T cells mediates durable and complete disease regression in some patients with metastatic cancer. The long-term survival and antitumor immunity of adoptively transferred $CD8^+$ T cells are dependent on the cells' metabolic fitness. Highly glycolytic T cells are short-lived after transfer and have impaired antitumor immunity, whereas T cells characterized by high FAO and SRC have greater long-term survival. Isolation and transfer of T cells with low mitochondrial membrane potential, as an indicator of improved metabolic fitness, was associated with better long-term *in vivo* persistence and an enhanced capacity to eradicate established tumors compared with cells with high mitochondrial membrane potential[53]. Such metabolic sorting could complement sorting based on conventional surface markers in identifying cells with the capacity for long-term survival and ongoing effector function after transfer.

Manipulation of $CD8^+$ T cell metabolism may also be of therapeutic benefit. CAR is a recombinant membrane receptor that recognizes a surface antigen on cancer cell targets and activates TCR signaling and an anticancer response. CAR expression has been associated with OXPHOS and longevity in $CD8^+$ T cells[118]. Costimulation of CAR T cells via the coreceptor 4-1BB leads to increased clinical efficacy and persistence of these cells[119]. T cells with a 4-1BB CAR have increased FAO, mitochondrial biogenesis and SRC[114], which may underpin their enhanced survival. In contrast, CAR T cells costimulated via CD28 are more effector-like and short-lived. CAR T cells that express both coreceptors may provide synergistic antitumor immunity.

Concluding remarks

The early pioneers of mitochondria research would be fascinated by the discoveries that have been made concerning mitochondria and immune-cell function. For immunologists, mitochondria

can be seen as the powerhouse of immunity, in addition to their role as the powerhouse of the cell. Future work will continue to reveal the functions of mitochondria in inflammation (**Box 1**), with a tantalizing array of therapeutic possibilities for inflammatory diseases and cancer.

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COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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